International Journal of Phytoremediation
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713610150

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Online Publication Date: 01 July 2007
To cite this Article: Zalesny Jr., Ronald S. and Bauer, Edmund O. (2007) 'Evaluation of **Populus** and **Salix** Continuously Irrigated with Landfill Leachate II. Soils and Early Tree Development', International Journal of Phytoremediation, 9:4, 307 - 323
To link to this article: DOI: 10.1080/15226510701476594
URL: http://dx.doi.org/10.1080/15226510701476594

International Journal of Phytoremediation, 9:307–323, 2007

ISSN: 1522-6514 print / 1549-7879 online
DOI: 10.1080/15226510701476594

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EVALUATION OF *POPULUS* AND *SALIX* CONTINUOUSLY IRRIGATED WITH LANDFILL LEACHATE II. SOILS AND EARLY TREE DEVELOPMENT

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Soil contaminant levels and early tree growth data are helpful for assessing phytoremediation systems.* Populus *(DN17, DN182, DN34, NM2, and NM6) and* Salix *(94003, 94012, S287, S566, and SX61) genotypes were irrigated with landfill leachate or municipal water and tested for differences in 1) element concentrations (P, K, Ca, Mg, S, Zn, B, Mn, Fe, Cu, Al, and Na) of a topsoil layer and a layer of sand in tanks with a cover crop of trees or no trees and 2) height, diameter, volume, and dry mass of leaves, stems, and roots. Trees were irrigated with leachate or water during the final 12 wk of the 18-wk study. Differences in most soil element concentrations were negligible ($P > 0.05$) for irrigation treatments and cover main effects. Phosphorous, K, Mg, S, Zn, Mn, Fe, and Al concentrations were greater in topsoil than sand ($P = 0.0011$ for Mg; $P < 0.0001$ for others). There was broad variation between genera and among clones for all growth traits. The treatment × clone interaction governed height, volume, and root dry mass, with (94012, SX61), (NM2, S566, SX61), and (S287, S566) exhibiting the greatest levels, respectively, following leachate application. Given the broad amount of variability among and within these genera, there is great potential for the identification and selection of specific genotypes with a combination of elevated phytoremediation capabilities and tree yield. From a practical standpoint, these results may be used as a baseline for the development of future remediation systems.

KEY WORDS: phytoremediation, poplar, willow, edaphic properties, tree volume, tree biomass

INTRODUCTION

Assessing the variability in remediation capabilities of *Populus* and *Salix* genotypes is a necessary component in the development of phytoremediation systems (Zalesny and Bauer, 2007). In addition, evaluation of soil contaminant levels and tree yield (*i.e.*, volume and biomass) are equally-important components. While the effects of soil contaminant levels on plant growth are well-documented (Marschner, 1995), there is a lack of knowledge about the correlation between *Populus* and *Salix* phytoremediation capabilities and tree productivity. It is logical to assert that the genotypes expressing the greatest phytoremediation levels are those with the best yield (Moffat, Armstrong, and Ockleston, 2001). However, an inverse

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relationship between these traits has been reported for *Populus* (Zalesny and Bauer, 2007) and *Salix* (Greger and Landberg, 1999; Klang-Westin and Eriksson, 2003). Nevertheless, given that genetic gain is proportional to variation and that there is broad amount of variability among and within these genera (Aravanopoulos, Kim, and Zsuffa, 1999; Eckenwalder, 1984), there is a great likelihood for successful identification and selection of specific genotypes with a combination of elevated phytoremediation capabilities and tree yield.

The importance of the interaction among soil nutrient levels, concentrations of nutrients sequestered, and subsequent tree productivity has been reported (Ericsson, Rytter, and Lindner, 1992). The need to consider all three factors is intuitive, because soil concentration levels provide a means of estimating the availability of nutrients, while phytoremediation and subsequent translocation into plant tissues is an insightful measure that helps researchers and resource managers match genotypes to specific nutrients (Marschner, 1995). Yield is important because tree establishment and subsequent development are requirements for successful phytoremediation, regardless of the contaminant (Zalesny *et al*., 2005c). Ideally, information about all three factors should support the selection of proper genotypes that can be grown under conditions of elevated element concentrations, while subsequently reducing soil contamination levels *via* translocation to biomass that can be harvested (Labrecque, Teodorescu, and Daigle, 1998). Likewise, researchers have gone beyond a simple examination of productivity and tested the effects of irrigation and fertilization on the productivity of *Populus* (Brown and van den Driessche, 2005; Coleman, Friend, and Kern, 2004; Coyle and Coleman, 2005) and *Salix* (Rytter and Hansson, 1996), which is similar to the utilization of landfill leachate as irrigation and fertilization in the current study.

Given the need for tree establishment on soils contaminated with landfill leachate (Bialowiec, Wojnowska-Baryla, and Hasso-Agopsowicz, 2003; Shrive, McBride, and Gordon, 1994), we seek knowledge about element concentrations in soils, along with data on early tree development. Such information will provide a baseline for future researchers in developing additional studies and for resource managers in need of cost-effective remediation technologies. The specific objectives of the current study were to irrigate *Populus* and *Salix* genotypes with landfill leachate or municipal water and to: 1) test for differences in element concentrations of a topsoil layer and a middle layer of sand in tanks that had a cover crop of trees or no trees and 2) test for differences in height, diameter, volume, and dry mass of leaves, stems, and roots. Our initial hypotheses were two-fold. First, element concentrations in the soil would differ based on treatment, cover, and horizon (*i.e*., treatment × cover × horizon interactions would exist). Second, variation in early developmental traits would be genotype-specific, following treatment application (*i.e*., genera and clones-within-genera would respond differently to treatments). We believe this information, along with remediation data, is necessary for the development of successful phytoremediation systems.

MATERIALS AND METHODS

Zalesny and Bauer (2007) provided a detailed description of clone selection, tree establishment, experimental design, and treatment application. In summary, the *Populus* clones and their genomic groups were DN34, DN17, DN182 (*P. deltoides* Bartr. ex Marsh × *P. nigra* L.) and NM2, NM6 (*P. nigra* × *P. maximowiczii* A. Henry). The *Salix* clones and

Table 1 Mean temperature, total precipitation, and total number of growing degree days (GDD) from June to October 1999 in Rhinelander, WI, USA (45.6°N, 89.4°W)

Month	Temperature (°C)	Precipitation (cm)	GDD
June	16	4.51	374
July	20	13.04	597
August	16	5.43	380
September	12	3.90	161
October	5	3.44	17

their genomic groups were 94003, 94012 (*S. purpurea* L.); S287 (*S. eriocephala* Michx.); S566 (*S. eriocephala* 28 × *S. eriocephala* 24); and SX61 (*S. sachalinensis* F. Schmidt).

The study was conducted outdoors and *ex situ* at the former city landfill in Rhinelander, WI, USA (45.6°N, 89.4°W). A summary of temperature, precipitation, and growing degree days across the experimental period is listed in Table 1. All cuttings, 25.4 cm long, were planted on June 14, 1999, in cattle watering tanks (600-L capacity) constructed of black polyethylene. A sheet of heavy-duty flat gray plastic was fitted to the tanks to reduce seepage of natural precipitation. At planting, incisions were made in the plastic and the cuttings were planted in paired rows according to genus, with the center line of the tanks used as a separator between *Populus* and *Salix* clones. Spacing between paired cuttings and between rows was 30 × 30 cm for all clones. The following three soil layers were used in the tanks: 1) 30 cm of topsoil obtained from the same source as the cover of the landfill, 2) a middle layer consisting of 30 cm of sand obtained from the same source as the sub-base of the landfill, and 3) 20 cm of washed 2- to 5-cm gravel as a bottom drainage layer. The soils of the landfill, classified as a Padus Loam (PaB), with 0 to 6% slopes, are considered to be well to moderately well drained with loamy deposits underlain by stratified sand and gravel glacial outwash. Soil characteristics from a depth of 0 to 12.7 cm to a depth of 88.9 to 152.4 cm range from 1.4 to 1.5 g cm^{-3} to 1.5 to 1.8 g cm^{-3} (moist bulk density), 1.5 to 15.2 cm h^{-1} to 15.2 to 50.8 cm h^{-1} (permeability), and 4.5 to 6.5 pH to 5.1 to 6.5 pH (soil acidity).

The treatments, of equal volume, consisted of leachate from the landfill (pH 6.3) and a control with application of water from the Rhinelander municipal water supply (pH 8.4). Also tested were the aforementioned genera and clones. Ten tanks were used for the following combinations: three tanks for leachate without trees, three tanks for leachate with trees, three tanks for municipal water with trees, and one tank for the overall control, municipal water without trees. For the tanks with trees, the cuttings were arranged in a nested split-plot design, with two treatments, two genera, five clones per genus, and three replications (tanks) per treatment. Treatments were arranged randomly per tank, while genera were considered whole plots because each genus was randomly isolated to a side of each tank. Clones-nested-within-genera were considered to be sub-plots and were randomly planted in two-tree plots.

All tanks were irrigated to field capacity with municipal water every other day for the initial 6 wk following planting. Over the remaining 12 wk of the study, tanks received two 75.7-L applications of leachate or municipal water during the first treatment week, two 37.9-L applications per week during treatment weeks 2 to 6, and two 56.8-L applications per week during treatment weeks 7 to 9. One application per week of 56.8, 37.9, and 18.9 L was applied during treatment weeks 10, 11, and 12, respectively. The volume of irrigation varied to simulate trends in natural precipitation events for northern Wisconsin (Table 1).

Sampling and Measurements

Leachate and municipal water. Zalesny and Bauer () described the concentration of the following elements in the leachate and municipal water: phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), boron (B), manganese (Mn), iron (Fe), copper (Cu), aluminum (Al), sodium (Na), and chloride (Cl), along with the sum total amount of each element applied.

Elements in soils. On November 16, 1999, two samples were collected from each tank using a 5-cm hand auger. One sample was collected from the aforementioned layer of topsoil and the other sample was collected from the middle layer of sand. A 25-mg sample from each collection was weighed and placed in clean laboratory jars. The samples were sent to the University of Wisconsin Soil & Plant Analysis Laboratory (Madison, WI, USA) for element analysis by inductively coupled plasma optical emission spectrometry (ICP-OES).

Tree height, diameter, volume, and biomass. Prior to plant harvest, tree height to the nearest 1.00 cm and diameter to the nearest 0.01 cm was determined for all trees. To reduce experimental error, height was measured from ground level to the base of the apical bud on the terminal shoot and diameter was measured at 10 cm above the soil surface. Volume (cm^3) was estimated using the generalized equation: $volume = diameter^2 \times height$, according to Avery and Burkhart (1994). Dry biomass to the nearest 0.01 g of leaves, stems, and roots was recorded during whole-plant sampling for element analysis (Zalesny and Bauer,).

Data Analysis

Elements in soils. Soil-related element data were subjected to analyses of variance according to SAS® (PROC GLM; SAS Institute, 2004) assuming a factorial design with fixed main effects for treatment (leachate and municipal water), cover (no trees and trees), and horizon (upper topsoil and lower sand) (Table 2). The following linear additive model was used in the analysis:

$$Y_{ijkl} = \mu + T_i + C_j + TC_{ij} + H_k + TH_{ik} + CH_{jk} + TCH_{ijk} + E_{(ijk)l}$$

where Y_{ijkl} = response variable to be analyzed, μ = overall mean, T_i = main effect of the ith treatment, C_j = main effect of the jth cover, TC_{ij} = effect of interaction between the ith treatment and the jth cover, H_k = main effect of the kth soil horizon, TH_{ik} = effect of interaction between the ith treatment and the kth soil horizon, CH_{jk} = effect of interaction between the jth cover and the kth soil horizon, TCH_{ijk} = effect of interaction between the ith treatment, the jth cover, and the kth soil horizon, and $E_{(ijk)l}$ = experimental error, NID (o,σ^2).

Tree height, diameter, volume, and biomass. Tree height, diameter, volume, and biomass data were subjected to analyses of variance according to SAS® (PROC GLM; SAS Institute, 2004) assuming a nested split-plot design with fixed main effects for treatment (leachate and municipal water), genus (whole plot; *Populus* and *Salix*),

Table 2 Degrees of freedom and expected mean squares from analyses of variance in experiments testing the effects of treatment (leachate and municipal water), cover (no trees and trees), and horizon (upper topsoil and lower sand) on element concentration of soils (Model I), along with treatment, genus (*Populus*, *Salix*), and clone (five per genus, see Materials and Methods) on tree height, diameter, volume, and biomass components (Model II)

Source: Model I	df	Expected mean squares	Source: Model II	df	Expected mean squares
Treatment	1	$\sigma^2 + 4\Phi_{\mathrm{T}}$	Treatment	1	$\sigma^2 + 5\sigma^2{}_{\mathrm{TGA}} + 30\Phi_{\mathrm{T}}$
Cover	1	$\sigma^2 + 4\Phi_{\mathrm{C}}$	Genus	1	$\sigma^2 + 5\sigma^2{}_{\mathrm{TGA}} + 30\Phi_{\mathrm{G}}$
Treatment × Cover	1	$\sigma^2 + 2\Phi_{\mathrm{TC}}$	Treatment × Genus	1	$\sigma^2 + 5\sigma^2{}_{\mathrm{TGA}} + 15\Phi_{\mathrm{TG}}$
Horizon	1	$\sigma^2 + 4\Phi_{\mathrm{H}}$	Tank (Treatment × Genus)	8	$\sigma^2 + 5\sigma^2{}_{\mathrm{TGA}}$
Treatment × Horizon	1	$\sigma^2 + 2\Phi_{\mathrm{TH}}$	Clone (Genus)	8	$\sigma^2 + 6\Phi_{\mathrm{GC}}$
Cover × Horizon	1	$\sigma^2 + 2\Phi_{\mathrm{CH}}$	Treatment × Clone (Genus)	8	$\sigma^2 + 3\Phi_{\mathrm{TGC}}$
Treatment × Cover × Horizon	1	$\sigma^2 + \Phi_{\mathrm{TCH}}$	Error	32	σ^2
Error	12	σ^2	Pooled Error	59	
Pooled Error	19				

and clone-nested-within-genus (sub-plot; *Populus*—DN17, DN34, DN182, NM2, NM6; *Salix*—94003, 94012, S287, S566, SX61) (Table 2). There were three replications (tanks) for each treatment. The following linear additive model was used in the analysis:

$$Y_{ijklm} = \mu + T_i + G_j + TG_{ij} + E_{(ij)k} + C_{(j)l} + TC_{i(j)l} + E_{(ijkl)m}$$

where Y_{ijklm} = response variable to be analyzed, μ = overall mean, T_i = main effect of the *i*th treatment, G_j = main effect of the *i*th genus, TG_{ij} = effect of interaction between the *i*th treatment and the *j*th genus, $E_{(ij)k}$ = whole-plot error, NID (o,σ^2), $C_{(j)l}$ = main effect of the *l*th clone nested within the *j*th genus, $TC_{i(j)l}$ = effect of interaction between the *i*th treatment and the *l*th clone nested within the *j*th genus, and $E_{(ijkl)m}$ = sub-plot error, NID (o,σ^2).

Analyses of covariance were conducted to test for the effect of cutting dry mass on all tree growth-related variables and cutting dry mass was not a significant covariate for any variable ($P > 0.05$). *Populus* genomic groups were differentiated according to single degree-of-freedom linear contrasts ($\alpha = 0.05$). Fisher's protected least significant difference (LSD) was used to compare means of main effects and interactions (Chew, 1976).

RESULTS

Elements in Soils

The topsoil contained greater amounts of each element than the sand and differences within each layer were dependent upon the treatment and the presence of trees. Treatment ($P = 0.0302$) and cover ($P = 0.0466$) main effects were significant for Na, while cover differed for Cu ($P = 0.0326$). The interaction between treatment and cover governed the concentration of Ca ($P = 0.0346$). Treatment and cover main effects and their interaction were negligible for all other elements ($P > 0.05$). The concentration of Na in the soils of the leachate and municipal water treatment was 49.79 ± 2.91 and 62.17 ± 4.11 mg kg^{-1}, respectively; while that for the cover treatment of trees and no trees was 61.57 ± 2.91 and 50.39 ± 4.11 mg kg^{-1}, respectively. Likewise, the soils exhibited a greater concentration of Cu with trees (7.38 ± 0.20 mg kg^{-1}) than without trees (6.54 ± 0.28 mg kg^{-1}). The Ca concentration for treatment × cover interactions was 1972.21 ± 128.25 mg kg^{-1} (leachate × trees), 1549.45 ± 128.25 mg kg^{-1} (leachate × no trees), 1721.66 ± 128.25 mg kg^{-1} (water × trees), and 2047.48 ± 222.14 mg kg^{-1} (water × no trees) ($LSD_{0.05,12} = 395.22$ mg Ca kg^{-1}, n = 6).

The main effect of horizon was significant for the concentration of P, K, Mg, S, Zn, Mn, Fe, and Al ($P = 0.0011$ for Mg, $P < 0.0001$ for others), but the interaction between treatment, cover, and horizon governed the concentration of P ($P = 0.0113$), K ($P = 0.0188$), and Mn ($P = 0.0446$). The concentration of most elements was significantly greater in the topsoil than in the middle layer of sand (Table 3). In addition, regardless of the treatment and cover combination, the concentration of P, K, and Mn was greatest in the topsoil (Figure 1). Overall, the mean concentration of P across treatments, covers, and horizons was 294.97 ± 21.33 mg kg^{-1}. The greatest P concentration was in the topsoil after treatment with municipal water but without trees. Similar results were expressed for K. However, the combination of leachate with trees, which was not different from other combinations, exhibited a similar concentration as that with water and no trees. Overall, the mean concentration of K across treatments, covers, and horizons was 456.54 ± 19.17

Table 3 Mean concentration of elements across leachate and water treatments in 30 cm of topsoil and 30 cm of a middle layer of sand (n = 10 for each) in tanks during an experiment testing clone-specific phytoremediation capabilities of *Populus* and *Salix*. All pairwise comparisons are different at $\alpha = 0.05$, except for Ca, B, Cu, and Na

	Concentration (mg kg^{-1})		
Element	Topsoil	Sand	Standard error
P	394.90	202.17	11.11
K	539.74	378.02	14.70
Mg	1890.30	1588.67	50.17
S	140.74	36.37	6.31
Zn	29.47	13.85	0.90
Mn	405.72	166.33	15.11
Fe	13639.07	8009.82	301.23
Al	9658.77	5170.08	234.06
Ca	1883.28	1762.12	111.07
B	11.65	12.33	0.48
Cu	6.94	6.97	0.25
Na	58.49	53.47	3.56

mg kg^{-1}. The concentration of Mn exhibited a similar trend as K, except that application of leachate and coverage with trees exhibited the greatest concentration of Mn, while that with water and without trees was similar but not different from the other combinations in the topsoil. Overall, the mean concentration of Mn across treatments, covers, and horizons was 283.99 ± 28.90 mg kg^{-1}.

Tree Height, Diameter, Volume, and Biomass

Tree growth varied greatly between *Populus* and *Salix*, with *Populus* exhibiting the greatest diameter, volume, and leaf dry mass and *Salix* exhibiting the greatest height and dry mass of stems and roots (Table 4). The genus main effect was significant for height ($P = 0.0308$), diameter ($P = 0.0013$), volume ($P = 0.0448$), and dry mass of the leaves ($P < 0.0001$), stems ($P = 0.0186$), and roots ($P = 0.0033$). The treatment main effect and the interaction between treatment and genus were negligible for all traits ($P > 0.05$).

Table 4 Height, diameter, volume, and biomass components (± standard error, n = 60) of trees during an experiment testing clone-specific phytoremediation capabilities of *Populus* and *Salix*. All pairwise comparisons are different at $\alpha = 0.05$

	Genus	
Trait	*Populus*	*Salix*
Height (cm)	99.74 ± 2.27	117.19 ± 2.26
Diameter (cm)	1.00 ± 0.02	0.79 ± 0.02
Volume (cm^3)	113.12 ± 5.94	85.28 ± 5.90
Leaf dry mass (g)	6.79 ± 0.78	1.16 ± 0.79
Stem dry mass (g)	17.38 ± 1.03	25.67 ± 1.03
Root dry mass (g)	4.49 ± 0.47	8.37 ± 0.47

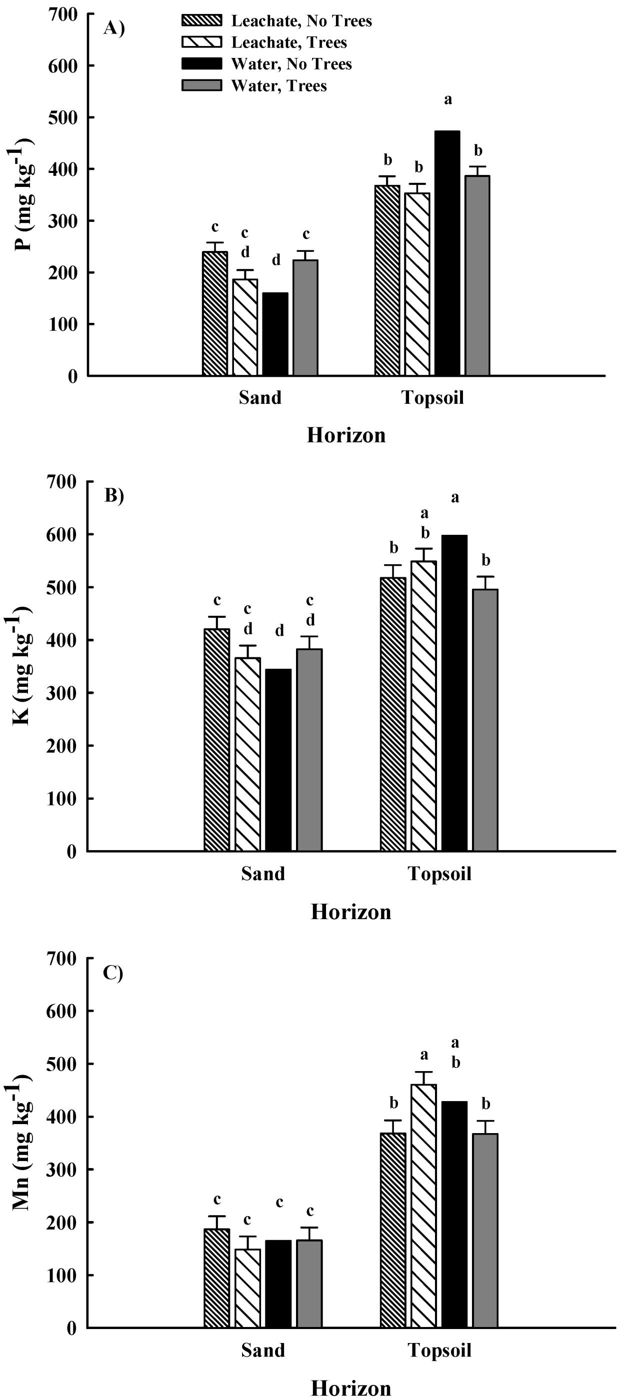

Figure 1 Concentration of P, K, and Mn for each combination of treatment (leachate and municipal water), cover (no trees and trees), and soil horizon (upper topsoil and lower sand) in tanks during an experiment testing clone-specific phytoremediation capabilities of *Populus* and *Salix*. Standard error bars represent one standard error of the mean, n = 3 trees for each three-way interaction, except for water and no trees where n = 1. Concentrations with different letters above the bars are different according to Fisher's protected least significant difference (LSD) ($\alpha = 0.05$, LSD = 55.93 mg P kg^{-1}, 73.99 mg K kg^{-1}, and 76.03 mg Mn kg^{-1}).

The main effect of clone was significant for height, diameter, volume, and dry mass of the stems and roots ($P < 0.0001$), but the interaction between treatment and clone governed height ($P = 0.0247$), volume ($P = 0.0084$), and root dry mass ($P = 0.0260$). Diameter and stem dry mass varied greatly among clones (Figure 2). The variation in diameter for *Populus* clones was not as broad as that for *Salix* clones, whereby S566 and SX61 exhibited the greatest diameter. However, the diameter was greater for NM clones than DN genotypes ($P = 0.0232$). Variation among clones for stem dry mass was greater than for diameter. Overall, *Salix* clone S566 exhibited the greatest stem dry mass, while clones 94012, S287, and SX61, along with *Populus* clone NM6, were in the second-best response group. Once again, NM clones outperformed DN clones ($P = 0.0171$). Overall, the mean diameter across clones was 0.89 ± 0.02 cm, while the mean stem dry mass across clones was 21.49 ± 1.06 g. In general, there was a lack of an advantage of using clones belonging to either genus. However, broad genotypic variation existed, with clone-specific responses to leachate and water treatments influencing height, volume, and root dry mass (Figure 3). Overall, the mean height across treatments and clones was 108.00 ± 2.68 cm, while the mean volume was 97.46 ± 5.76 cm^3, and the mean root dry mass was 6.39 ± 0.51 g.

DISCUSSION

The main goals of this study were to irrigate *Populus* and *Salix* genotypes with landfill leachate or municipal water and to 1) test for differences in element concentrations of a topsoil layer and a middle layer of sand in tanks that had a cover crop of trees or no trees and 2) test for differences in height, diameter, volume, and dry mass of leaves, stems, and roots. The results should be interpreted considering the treatments were applied over one growing season. Changes in the specific differences and/or the magnitude of differences most likely would exist in a longer-term study. In addition, the lack of nitrogen data should be considered. Nitrogen is a key element for tree growth and development, along with being important for determining application rates for landfill leachate used as irrigation. Nevertheless, applying leachate or municipal water, along with establishing trees or not having trees, did not have a significant effect on most element concentrations in the soil. The concentration of P, K, Mg, S, Zn, Mn, Fe, and Al, however, was greater in the topsoil than in the middle layer of sand. Most likely, these differences were due to greater initial water holding capacity and cation exchange capacity (CEC) in the topsoil relative to the sand. Overall, repeated leachate treatments affected the topsoil more than the sand. Increased element concentrations in the topsoil should promote tree growth, because most rooting occurs in the upper 30 cm of soil. Furthermore, the broad clonal variation in growth traits between genera and among clones-within-genera support the need for further testing and selecting of specific clones for various phytoremediation needs. The treatment $\times$ clone interaction governed height, volume, and root dry mass, while leachate did not affect the other growth traits. Overall, *Salix* clones 94012 and SX61 had the greatest height, *Populus* clone NM2 and *Salix* clones S566 and SX61 exhibited the greatest volume, and *Salix* clones S287 and S566 had the greatest root dry mass, following leachate application.

Elements in Soils

In general, treatment and cover main effects and their interaction did not affect element concentrations in the soils. However, the soil horizon main effect governed the concentration

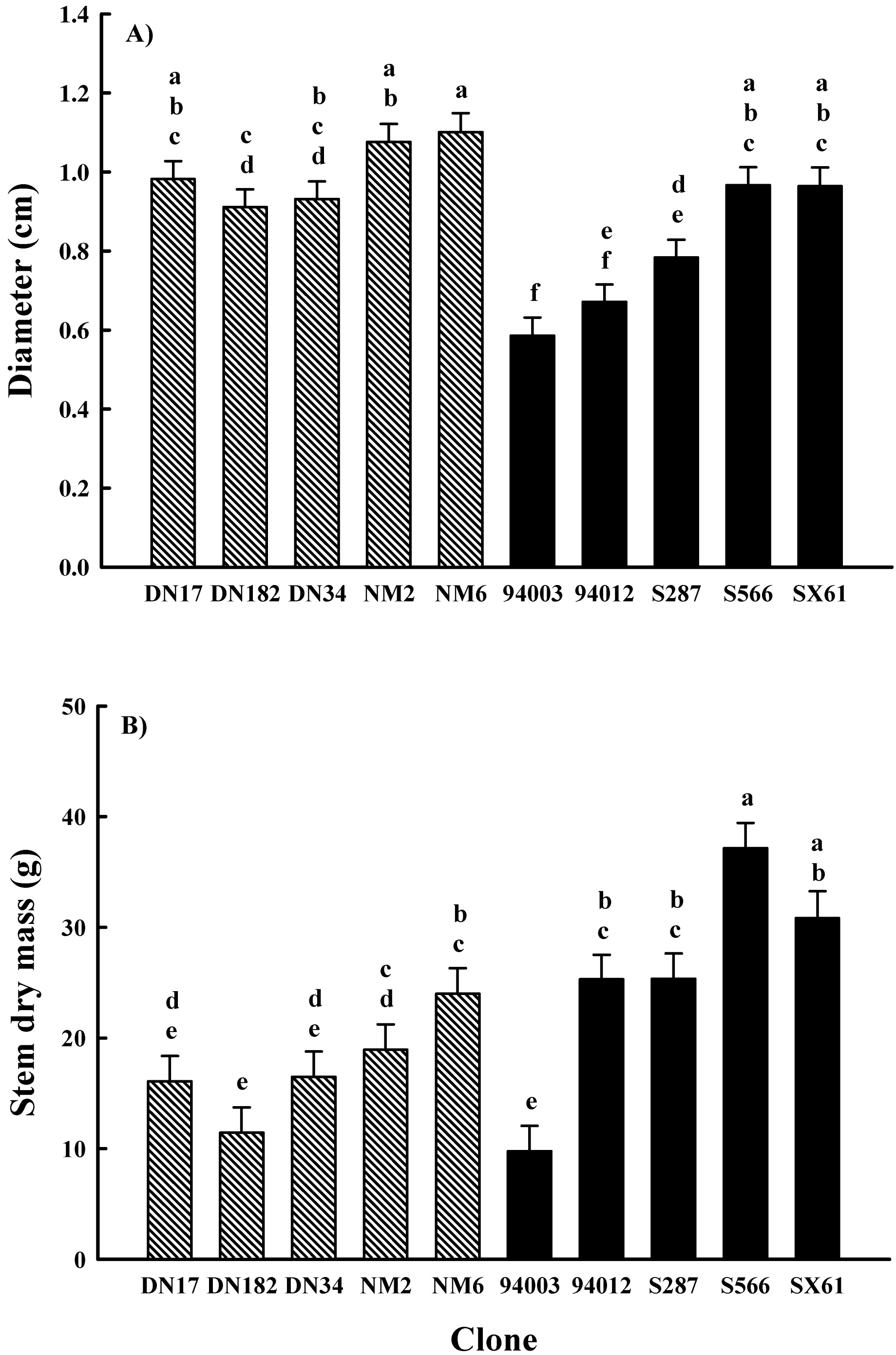

Figure 2 Diameter and stem dry mass across leachate and water treatments for *Populus* (dashed bars) and *Salix* (black shaded bars) clones during an experiment testing genotype-specific phytoremediation capabilities. Standard error bars represent one standard error of the mean, n = 12 trees for each clone. Traits with different letters above the bars are different according to Fisher's protected least significant difference (LSD) [$\alpha = 0.05$, LSD = 0.15 cm (diameter) and 7.48 g (stem dry mass)].

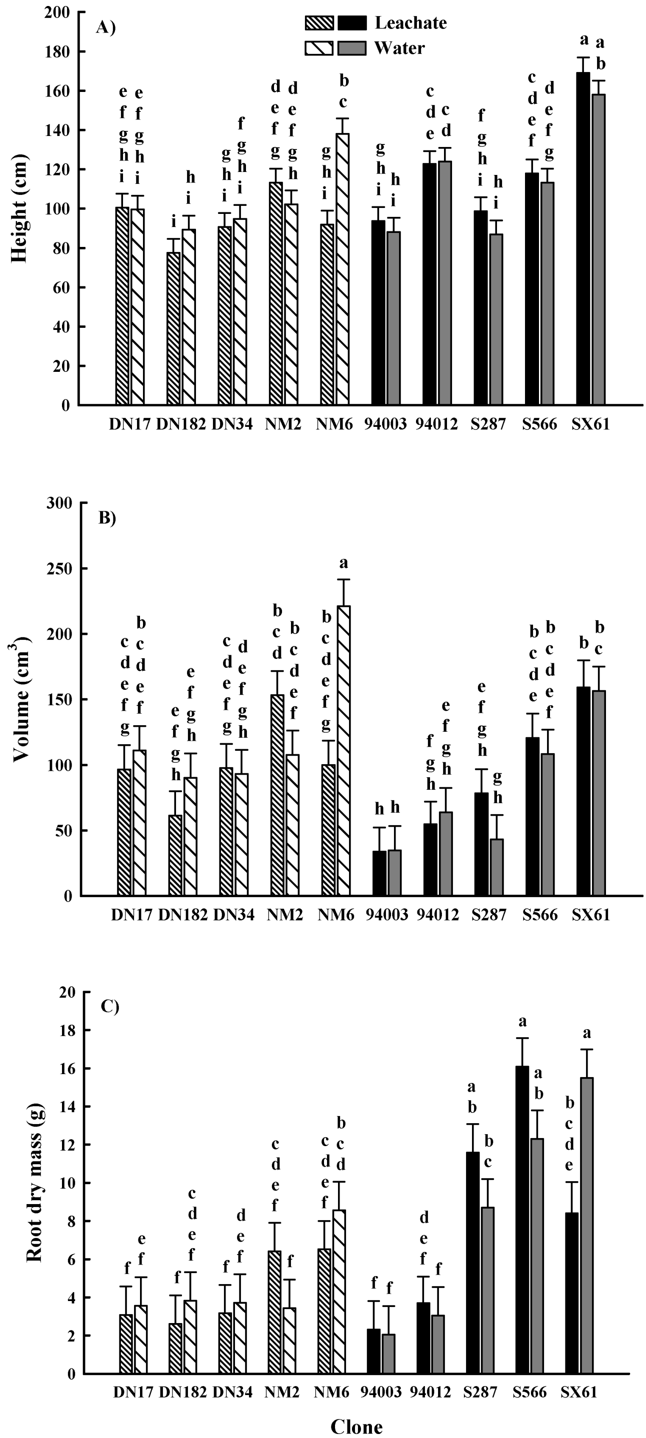

Figure 3 Height, volume, and root dry mass for each combination of treatment (leachate and municipal water) and clone during an experiment testing genotype-specific phytoremediation capabilities of *Populus* (dashed bars) and *Salix* (shaded bars). Standard error bars represent one standard error of the mean, n = 6 trees for each interaction. Traits with different letters above the bars are different according to Fisher's protected least significant difference (LSD) [$\alpha = 0.05$, LSD = 23.20 cm (height), 60.62 cm^3 (volume), and 4.87 g (root dry mass)].

of Mg, S, Zn, Fe, and Al, with the topsoil exhibiting equal to nearly four-fold greater concentrations than the sand for these elements. The Mg, S, and Fe concentrations (1890.30 $\pm$ 50.17, 140.74 $\pm$ 6.31, 13639.07 $\pm$ 301.23 mg kg^{-1}, respectively) in the topsoil were greater than those in another phytoremediation study (260, 19, 23.8 mg kg^{-1}, respectively) testing *Populus* clone DN34 for uptake and transformation of 2,4,6-trinitrotoluene (TNT) (Thompson, Ramer, and Schnoor, 1998). In addition, the Zn concentration (29.47 $\pm$ 0.90 mg kg^{-1}) in the topsoil was greater than that of Thompson *et al*. (1998) (1.2 mg kg^{-1}), but less than that of other reported studies (185.4 $\pm$ 12.9 to 770 $\pm$ 64 mg kg^{-1}) (Boye, 2002; Landberg and Greger, 1996; Shen *et al*., 2002). Nevertheless, despite a general discrepancy between soil element concentrations and plant nutrient requirements, along with an inverse relationship between solubility of Zn and Fe with pH (Marshner, 1995), the concentration of these elements in the leaves, stems, and roots of our genotypes (Zalesny and Bauer, 2007) were similar to those in the aforementioned studies.

Our results were intuitive, given the increased water-holding capacity and CEC of the topsoil relative to the sand, which has limited ionic binding capacity and negligible contribution to the CEC (Foth, 1990; Havlin *et al*., 2005). Substantial amounts of observable organic matter in the topsoil relative to the sand likely contributed to greater adsorption of elements in the upper layer of soil (Boye, 2002; Dickmann *et al*., 2001; Marschner, 1995), along with lower concentrations of heavy metals and other elements in the plant tissues (Zalesny and Bauer, 2007) than were reported previously.

In addition, despite contrasting pH of the leachate (6.3) and municipal water (8.4), neither soil acidity nor alkalinity limited tree height, diameter, volume, and/or biomass. There was extensive variation among and within genomic groups for these traits, which likely was due to the different pH levels contributing to genotype $\times$ soil interactions. Conditions hindering growth and development have been reported at levels of pH < 5 (Timmer, 1985; van den Burg, 1980; van den Burg and Schoenfeld, 1978), which were not reached in the current study. Likewise, there were no visual symptoms of the Fe-deficiency chlorosis associated with elevated pH for the water treatments (Dickmann *et al*., 2001), even though the municipal water pH level was outside the optimal range (pH 5.0 to 7.5) for the enhanced productivity of *Populus* and *Salix* (Stanturf *et al*., 2001; Timmer, 1985). In contrast, such chlorosis was reported for a clone of *P. trichocarpa* Torr. & Gray (western black poplar) and an F_1 hybrid between *P. maximowiczii* and *P.* $\times$*berolinensis* (K. Koch) Dippel [*laurifolia* $\times$ *nigra*] (poplar) subjected to similarly-elevated pH levels (Heilman, 1992; van den Burg, 1980).

Moreover, to enhance the metal phytoextraction capabilities of similar *Populus* and *Salix* genotypes, future studies should test the effects of a chelating agent, such as ethylenediaminetetraacetic acid (EDTA), that would make the metals soluble and enhance their availability to the tree roots. Testing the interactions between EDTA and specific genotypes would also be meaningful. Other researchers have used EDTA and shown elevated phytoextraction of metals compared with control treatments (Kirkham, 2000; Lombi *et al*., 2001; Shen *et al*., 2002). The primary concern with such future studies is the leaching and subsequent dispersing of metal contaminants into groundwater aquifers, which must be considered before field-scale testing and/or deployment (Angle and Linacre, 2005).

The contrasting soils accounted for the element concentrations in the horizons, regardless of treatment or cover. Differences in soils have contributed substantially to element concentrations in plantations and forest stands (Bockheim and Crowley, 2002;

Labrecque *et al.*, 1998). Our first hypothesis was rejected for all elements except P, K, and Mn, where there was a treatment × cover × horizon interaction. The P, K, and Mn concentrations (394.90 ± 11.11, 539.74 ± 14.70, and 405.72 ± 15.11 mg kg^{-1}, respectively) in the topsoil were greater than those used previously (24, 180, and 4.8 mg kg^{-1}, respectively) (Thompson *et al.*, 1998). The element concentrations were greatest in the topsoil, but the most favorable treatments varied among elements. The greatest P concentration was with the combination of water and no trees, while the K and Mn concentration in the topsoil was greatest and similar to the following combinations: leachate + trees and water + no trees. Given greater than 1% organic matter in the topsoil and a reported increase in soil P concentration as pH elevates from 6 to 8 (Marschner, 1995), the higher soil P concentration with water and no trees may have been the result of the greater pH in the municipal water relative to the leachate. In addition, the P in the municipal water may have functioned as a fertilizer for the trees, which was illustrated by the difference in P levels between the covers of no trees and trees. Unfortunately, P levels in the leachate and water were not available, so comparisons with similar known P fertilization levels could not be made. However, it was reported that additions of essential elements increased the root proliferation and subsequent root length of northern hardwood trees, including *Populus grandidentata* Michx. (bigtooth aspen) (Pregitzer, Hendrick, and Fogel, 1993).

We speculate that increased soil aeration from the disturbance by tree roots may have led to increased microbial activity in the rhizosphere (Landmeyer, 2001; Marschner, 1995; Yateem *et al.*, 2000). Associated root exudates in the soils likely contributed to greater K and Mn concentrations in the topsoil irrigated with leachate and having trees versus no trees, along with an inverse response for topsoil irrigated with water (*i.e.*, concentrations were greatest with water and no trees). From a phytoremediation perspective, exudates are compounds produced/released through tree roots that are associated with phytodegradation of organic compounds (Anderson, Guthrie, and Walton, 1993; Burken and Schnoor, 1996; Jordahl *et al.*, 1997). The tree roots and their exudates in the current study may have contributed to greater availability of K and Mn in the topsoil relative to the sand, given the elevated concentrations in the leachate compared with the water. Unfortunately, our objectives did not support testing of this hypothesis. However, further tests are warranted and such experiments should focus on element concentrations at the interface between the tree roots and the root exudates (*i.e.*, the rhizosphere) in different soils and under different treatment regimes.

Tree Height, Diameter, Volume, and Biomass

There were broad differences in tree growth traits among the genotypes studied. Similar genotypic variation has been reported for the establishment of *Populus* and *Salix* on contaminated soils (Greger and Landberg, 1999; Jordahl, 1997; Moffat *et al.*, 2001; Perttu and Kowalik, 1997). At the genus level in our study, *Populu*s exhibited the greatest diameter, volume, and leaf dry mass, while *Salix* had greater height and dry mass of the stems and roots. Moreover, our second hypothesis was upheld for variation in height, volume, and root dry mass. Genera and clones-within-genera responded differently to leachate and water treatment. These results corroborated those of Moffat *et al.* (2001), who reported 50% greater biomass from a *P. trichocarpa* × *P. deltoides* hybrid ("Beaupré") relative to a pure *P. trichocarpa* clone ("Trichobel"), when treated with sewage sludge and effluent. However, despite extensive variation in height and volume across genotypes, differences in treatment responses within clones were negligible except for *Populus* clone NM6, which responded better to water treatment than leachate. There were similar responses for root dry mass, where within-clone differences were negligible except for *Salix* clone SX61, which responded best when irrigated with water. The lack of within-clone treatment advantages for biomass differed from those previously reported for *Salix*, wherein biomass increased following irrigation with municipal waste products (Hasselgren, 1998; Labrecque *et al.*, 1998).

Breeding and selecting *Populus* and *Salix* with traits suitable for phytoremediation is an ongoing concept in need of continual investigation (Bañuelos *et al.*, 1999; Landberg and Greger, 1996; Perttu and Kowalik, 1997; Zalesny *et al.*, 2005c). In the current study, from a breeding and genetics standpoint, there were three important genotypic responses. First, DN *Populus* clones performed similarly when irrigated with leachate or water. Recently, researchers have reported below-average growth performance (*i.e.*, rooting, diameter, and height) of traditional DN genotypes such as clones DN34 and DN182 (Riemenschneider *et al.*, 2001; Zalesny and Wiese, 2006; Zalesny *et al.*, 2005a), which has led to the consideration of less deployment of these DN clones, especially in the North Central United States. However, the results of the current study were important because, along with phytoremediation capabilities (Zalesny and Bauer, 2007) and promising results from recent breeding efforts (Neil Nelson, USFS NCRS, William Berguson, University of Minnesota NRRI, personal communication) DN clones appear to be suitable genotypes for the cleanup of leachate with similar contaminant levels and, possibly, of other contaminants. Although the inverse relationship between productivity and phytoremediation capability did not exist for two other *Populus* genotypes (Moffat *et al.*, 2001), results similar to ours have been reported for *Salix* (Greger and Landberg, 1999). Klang-Westin and Eriksson (2003) tested *S. viminalis* and reported stands exhibiting the greatest biomass had less stem cadmium (Cd) concentrations than those from low-productivity stands. This inverse relationship supports the need to recognize and work toward the proper balance between productivity and remediation capability so that both components of the phytoremediation system are effective. Overall, our previous assertion of the necessity to select proper genotypes, based on the desired end-use of the trees, was corroborated. Second, *Populus* clones NM2 and NM6 exhibited contrasting responses to leachate or water treatment, despite both having *P. nigra* × *P. maximowiczii* parentage. Given a general lack of differences for height, volume, and root dry mass, these observations must be interpreted cautiously. Generally, clones NM2 and NM6 have performed similarly for above- and below-ground traits (Riemenschneider *et al.*, 2001; Zalesny and Wiese, 2006; Zalesny *et al.*, 2003). Researchers have selected and utilized both clones almost interchangeably. Therefore, the different responses of clones NM2 and NM6 led us to assert that clonal selection for phytoremediation, regardless of the contaminant, should not be based solely on previous above- and below-ground growth, but on the results of much-needed intensive genotypic screening prior to large-scale use. Third, *Salix* clones S287 and S566 exhibited responses favoring leachate irrigation over water. Despite a lack of differences, cautious interpretation led us to assert there was an advantage of using clones with *S. eriocephala* parentage. Overall, researchers and resource managers should invest substantial resources at the strategic level (*i.e.*, selection of parental species), in addition to selecting specific clones for operational use (*i.e.*, selection within genomic groups) (Zalesny *et al.*, 2005b).

ACKNOWLEDGMENTS

We thank Jud Isebrands (Environmental Forestry Consultants, LLC) and Don Riemenschneider (USFS) for advice during the planning and execution of the study, along with Timothy Volk (SUNY ESF) for supplying the *Salix* cuttings. We are indebted to Jill Zalesny (Iowa State University) for pivotal discussions about the interactions among soil science, plant physiology, and plant genetics. In addition, we are grateful to Steven Rock (US EPA) for insightful technical reviews and suggestions. Also, we appreciate the review of earlier versions of the manuscript from Robert Bridges, Mark Coleman, David Coyle, Deahn Donner, Neil Nelson, Adam Wiese, and Jill Zalesny.

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